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REVIEW

NOVEL INSIGHTS IN ADVANCED THYROID CARCINOMA: FROM MECHANISMS TO TREATMENTS

Development of 3D organoid models to study aggressive thyroid cancers

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Abstract

Thyroid cancer (TC), particularly aggressive forms such as poorly differentiated thyroid carcinoma and anaplastic thyroid carcinoma, presents considerable clinical challenges due to limited treatment options and suboptimal outcomes. Organoid models, derived from patient samples or pluripotent stem cells (PSCs), offer a robust framework for elucidating the biology of these malignancies. Recent advancements in patient-derived tumor organoid (PDTO) methodologies have facilitated more accurate representations of TCs, encompassing the heterogeneity of the disease and mechanisms of therapeutic resistance. PSC-derived models have further enabled the investigation of fundamental driving mechanisms behind thyroid carcinogenesis. This review highlights the progress made in the development of TC organoids, focusing on their utility in studying aggressive subtypes. We discuss innovative techniques for creating PDTOs and their applications in replicating essential features of the tumor microenvironment (TME), analyzing tumor progression, conducting drug screenings, and developing personalized therapeutic strategies tailored to individual patients. While PDTOs have become the predominant model for TC research, PSC-derived organoids provide insights into early carcinogenic events and mutation-specific processes that are often inaccessible in established tumors. Through these advancements, we emphasize the critical role of organoid models in bridging the divide between fundamental research and clinical application, offering a promising avenue for uncovering novel insights into TC biology and enhancing therapeutic strategies.

Keywords: thyroid cancer; organoids; *in vitro* models

Introduction

Thyroid cancer (TC) is the most common endocrine malignancy, with increasing incidence over recent decades (1). Follicular cell-derived neoplasms include well-differentiated, high-grade differentiated, and poorly/undifferentiated thyroid carcinomas. Well-differentiated thyroid carcinoma (WDTC) comprises 90% of thyroid malignancies, including papillary thyroid carcinoma (PTC) (~80% of cases),

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follicular thyroid carcinoma (FTC) (~10%), and rare oncocytic (Hürthle cell) thyroid carcinoma (2). PTC and FTC have favorable prognoses (99% and ~90% survival rates, respectively), but ~20% of PTC cases become resistant to conventional therapies such as surgery and radioactive iodine (RAI), leading to poorer outcomes (3, 4). The advanced forms of TC include poorly differentiated thyroid carcinoma (PDTC) and anaplastic thyroid carcinoma (ATC). These subtypes are characterized by a dedifferentiated state, aggressive clinical behavior, rapid disease progression, and resistance to standard therapeutic approaches. Although PDTC and ATC together account for only 2–5% of all TCs, they are responsible for 20–50% of TC-related deaths and have a poor prognosis, with a 10–15% 1-year survival rate following diagnosis (1, 5, 6). Another type of TC – medullary thyroid carcinoma – arises from parafollicular C cells and is a rare neoplasia (<5% prevalence) (7), which, due to its distinct cell origin, will not be discussed in this review.

Hallmark mutations in WDTCs – such as $BRAF^{V600E}$ in PTC and $NRAS^{Q61R}$ in FTC – are linked to tumor development, increased growth, invasiveness, and resistance to apoptosis (8, 9). Additional driver mutations/rearrangements involving tyrosine kinase receptor genes (*RET*, *ALK*, and *NTRK*) are also implicated in PTC formation, while *PI3KCA* and *CTNNB1* mutations contribute to PTC progression (2, 5, 8). FTC frequently involves *NRAS* mutations (17–57% of cases) and *PAX8-PPARγ* fusion (12–53% of cases) (5, 7). The progression from WDTCs to more aggressive forms is associated with the acquisition and accumulation of additional genetic and epigenetic alterations, which is associated with a worse prognosis (8, 10). This transition is commonly characterized by mutations in *TP53*, *TERT* promoter, and in genes involved in the *PI3K/AKT/mTOR* pathway, which also contribute to dedifferentiation, enhanced metastatic potential, and therapy resistance, including refractoriness to RAI (2, 11, 12).

Despite the strong evidence that these mutations not only initiate TC formation but also drive the progression to more aggressive forms, investigating this complex and multistep process is challenging, as current models are not able to completely recapitulate the intrinsic molecular and cellular dynamics, highlighting the need for more advanced systems to study the aggressive phenotypes.

Traditional 2D cell cultures and animal models have provided insights into TC drivers but fail to capture TME interactions and heterogeneity (13). Recent advances in stem cell technology and three-dimensional (3D) organoid models are overcoming these limitations. Healthy thyroid organoids from human pluripotent stem cells (hPSCs) have shown the capacity to mimic thyroid gland development and function (14). Cancer organoids can be created by introducing genetic alterations into healthy organoids or by culturing tumor-derived samples (15, 16, 17), enabling tumor initiation, progression, and drug discovery studies (17, 18, 19). PDTOs preserve individual tumor features, allowing patient-specific therapeutic response testing and biomarker identification (20, 21).

This review explores recent progress in the use of 3D organoid models in TC basic and translational research, focusing on TC formation and progression, and their applications in identifying therapeutic targets and personalized therapies for aggressive TCs.

Organoids and 3D models' overview

By closely simulating the *in vivo* cellular microenvironment, 3D cell culture technology offers a powerful tool to bridge the limitations of 2D culture systems and the complexity and ethical aspects of animal models. Among these, spheroids and organoids have emerged as the most promising and versatile 3D culture systems due to their ability to accurately replicate the cellular heterogeneity and pathophysiological features of human cancers (22). Organoids are complex *in vitro* structures that replicate the key features of organs, including cellular complexity, structural architecture, and functional attributes (23). They can be derived either from pluripotent stem cells (embryonic stem cells; ESCs, or induced pluripotent stem cells; iPSCs), adult stem cells, tissue progenitors, or some differentiated cells such as hepatocytes and cholangiocytes (24). The pioneering development of organoids was accomplished by Clevers's group through the use of single leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5+) intestinal stem cells, isolated from mice. These stem cells, naturally residing in the crypts of the small intestine, were cultured to form small intestinal organoids. The process began with the emergence of cystic spheroids, which progressively formed crypt-like buds. Within 2 weeks, these structures evolved into complex, self-organizing 'mini-guts' that mimicked the crypt-villus architecture of the native intestine (25). Since then, this technology has been adopted and extended to generate a wide array of *in vitro* human organ models (extensively reviewed by Tang *et al.* (26)). Beyond their ability to mimic native organ functions, organoids offer several additional advantages that have sustained scientific interest and driven widespread enthusiasm for their use, including the capacity of being cultured for extended periods while preserving genetic stability. They are also amenable to cryopreservation and genetic manipulation, and can be passaged multiple times without losing their key characteristics (23, 27).

In most organs, the presence of resident stem cells has been clearly demonstrated, and their role in organoid culture establishment and maintenance is well-defined

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(25). In contrast to the intestine, thyroid tissue does not show a clear population of adult stem cells. Clevers's team demonstrated a lack of resident stem cell populations within adult mouse thyroid and organoids derived from adult mouse thyroid tissue using single-cell RNA sequencing (scRNAseq) (28). However, they identified a small cluster of proliferative thyroid cells that closely resembled mature thyroid follicular cells but exhibited lower expression levels of thyroid maturation markers such as thyroglobulin (Tg). Furthermore, a spatiotemporal atlas of the human thyroid during the first and second trimesters of pregnancy also did not identify a resident stem cell population in the thyroid (29). As hypothesized by Clevers's study, the 'progenitor-like' thyroid cells may be the population responsible for supporting the expansion and maintenance of mature thyroid organoids during successive passaging.

Compared to organoids, spheroid models are less complex systems; however, their utility has been shown by researchers studying TC, particularly for investigating cancer cell behavior and for high-throughput drug screening (HTS) (15, 30). Spheroids are easier and quicker to generate and consist of simple spherical aggregates of unique or multiple cell types (15, 22, 27, 30). They can be cultured either in suspension, without extracellular matrix (ECM) support, or embedded within an ECM that serves as a scaffold to promote cellular organization, proliferation, and interaction (15, 27). Scaffold-free methods are commonly used, as they are relatively simple, inexpensive, and rapid for generating spheroids (27). Furthermore, spheroids, in most cases, do not replicate organ-specific functions and display relatively limited structural and histological complexity compared to native organs or tumors (31). Unlike spheroids, organoids cannot be generated in suspension cultures, as their development requires a suitable ECM and specific differentiation or growth factors. These components are essential to support the 3D organization and functional maturation necessary to recreate organ-like structures that mimic human organ physiology (31). Several ECMs are commercialized for the generation of organoids. Among them, Matrigel (MTG) is the most widely used and has demonstrated its effectiveness in supporting cell expansion, differentiation, and self-assembly for various types of organoids (32). Nevertheless, MTG is poorly defined, and its composition varies from batch to batch, making it challenging to identify the precise signals that regulate stem cell differentiation and maintenance. In addition, MTG has limited tunability and reproducibility (33). Alternative matrices to MTG have been developed and have shown promise in supporting cell differentiation and self-assembly into 3D structures (reviewed by Gan *et al.* (33)). Each type of matrix offers distinct advantages and limitations, requiring careful selection tailored to the specific organ and experimental requirements.

Cancer organoid and spheroid models have been widely employed by researchers around the world to explore critical aspects of both early and advanced stages of TC. Several strategies have been developed to generate these models, including the introduction of tumorigenic alterations into PSCs and in non-diseased organoids (17). Another common approach involves dissociating TC tissue into single cells and culturing them within a scaffold-based ECM under appropriate conditions to foster the formation of 3D structures (34). Cancer organoids and spheroids are often used interchangeably in the literature, but they differ in important aspects, as described above (27, 31). As proposed by others, a more appropriate designation to encompass both cancer organoids and spheroids is tumoroids. This term is particularly fitting given that the word organoid typically refers to a miniature, organ-like structure that recapitulates both the architecture and function of a specific organ. In contrast, cancer tissues generally lack the specialized physiological structure and function characteristic of healthy organs. Instead, they are defined by abnormal growth, structural disorganization, and aberrant cellular behavior. Therefore, referring to these models as tumoroids more accurately reflects their pathological origin and biological properties. Nevertheless, in this review, we will use the designation cancer organoids or spheroids as stated by the studies.

Current status of thyroid cancer organoid models

Organoid models have revolutionized cancer research by offering 3D *in vitro* platforms that recapitulate human tissues' cellular architecture and functionality. In the field of TC, where aggressive forms present significant clinical challenges, the development of TC organoids can provide a powerful tool to investigate tumor biology, test therapeutic strategies, and explore patient-specific targeted therapy. Here, we will discuss the technical aspects, main findings, and pros and cons of the studies that have successfully generated TC organoids from both patient-derived tissues and PSCs.

Thyroid cancer tissue-derived models

The first tissue-derived TC organoid models were developed with the main application of studying specific tumor behaviors and performing drug screening. Saito *et al.* (2018) established a thyroid organoid culture system from mice that presented most of the essential thyroid functions (35). Isolated thyroid cells from 4- to 10-week-old C57BL/6 mice cultured in MTG using a conditioned growth medium resulted in TSH-responsive thyroid organoids capable of secreting thyroid hormone (TH) (35). To model TC, thyroid organoids derived from *TP53*-deficient ($TP53^{KO}$) mice

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were further modified to carry the *NRAS*^{Q61R} full-length mutation. Following subcutaneous xenotransplantation, the modified organoids formed TC-initiating cells that exhibited reduced Tg expression and histological features consistent with PDTC (35). In humans, while each mutation independently occurs in a significant subset of PDTCs, the simultaneous presence of both TP53 and *NRAS*^{Q61R} is rare but still probable (8). *NRAS*^{Q61R} mutations alone often drive the development of follicular-patterned carcinomas. However, the addition of a secondary mutation, such as *TP53*, significantly enhances tumor aggressiveness, potentially leading to ATC. This progression underscores the critical role of *TP53* loss in fostering more aggressive tumor phenotypes. This study marked the development of the first TC organoid model, providing a valuable platform to investigate thyroid carcinogenesis (35).

Recognizing the need for models that could more accurately reflect the complexity of patient-specific tumor biology, subsequent research efforts shifted toward the development of PDTO systems. The following studies illustrate how PDTOs could become a key tool for understanding patient-specific tumor behavior, helping to find new genes/pathways with prognostic and therapeutic significance, tumor initiation markers, or new forms of evaluating prognosis (36, 37, 38, 39, 40, 41, 42, 43). In addition, they constitute a powerful tool to perform drug screening for precision treatment for each patient and testing of new compounds with antitumoral potential (44, 45). Studies have also used PDTOs to explore the impact of exposure to different compounds found in the environment, such as endocrine disruptors (46, 47). Another important clinical aspect is the use of PDTOs to study drug resistance in aggressive forms of TC and provide insights into better management of the disease (42). In the following section, we discuss the most significant studies involving PDTOs.

Well-differentiated TCs are usually treated with thyroidectomy, often followed by RAI therapy to eliminate residual thyroid cells (1). However, advanced TCs with distant or local metastases, particularly those refractory to RAI, require alternative treatments. In such cases, targeted therapies are made depending on the tumor's molecular profile. FDA-approved options include antiangiogenic drugs such as sorafenib, lenvatinib, vandetanib, and cabozantinib, as well as targeted agents such as selective RET inhibitors (selpercatinib) for RET fusions/mutations, NTRK inhibitors (entrectinib, larotrectinib) for NTRK fusions, and the BRAF/MEK inhibitor combination (dabrafenib/trametinib) for *BRAF*^{V600E} mutations (1). 3D models have proven essential for developing targeted therapies and identifying genetic markers for drug development. Chen *et al.* (2021) expanded the application of PDTOs from PTC by establishing a long-term culture system capable of recapitulating both the histopathological and genomic features of the parental tumors (48). TC cells from PTC patients were cultured in MTG for at least 21 days in a supplemented growth medium, and PTC-like thyroid organoids were obtained. Through drug sensitivity assays, the authors demonstrated that these organoids could reliably reflect patient-specific responses to anticancer drugs, highlighting the role of estrogen receptor α (*ERα*) in promoting tumor proliferation. However, direct comparisons between organoid drug responses and patients' clinical outcomes could not be assessed. In a following study, the same group generated PTC organoids harboring the *BRAF*^{V600E} mutation to investigate the efficacy of BRAF inhibitor-based combination therapies (34). Using the same strategy to generate PDTOs, the authors tested various drugs targeting components of the RAS/RAF/MEK/ERK pathway and evaluated drug responses using different combinations of therapies. While BRAF inhibitors alone showed limited efficacy in treating *BRAF*^{V600E}-mutant organoids, combination therapies with MEK inhibitors, receptor tyrosine kinase inhibitors, or chemotherapeutic agents significantly improved treatment outcomes (34). The authors made a valuable observation regarding variability in drug responses among *BRAF*^{V600E} PDTOs and differences within the same individual. Considering the pronounced heterogeneity within tumor samples, the potential for intraindividual variation in drug response is significant. Using whole-genome sequencing on both fresh tumor tissue and PDTOs, they demonstrated that there are no significant differences in the exome, indicating that organoid culture does not induce genome instability. However, the transcriptome, epigenome, and cell heterogeneity status between the cancer tissue and derived PDTOs remain to be determined, and whether these organoids faithfully maintain the molecular characteristics over time of the original tumor is still an important question to be answered.

Recent studies have also generated models for more aggressive forms of TCs, broadening our understanding of therapeutic responses across these types of tumors. Hu *et al.* (2024) developed a 3D ATC spheroid culture system by seeding dissociated ATC cells into AggreWell plates to form aggregates, or into low-attachment plates for floating culture (15). On day 14, the spheroids were embedded in MTG, providing ECM support to maintain structural integrity for extended culture. These ATC spheroids mimicked patient-specific tumor features, including histological architecture, cohesive (*BRAF*^{WT}) and discohesive (*BRAF*^{V600E}) morphologies, and transcriptional profiles that more accurately reflected parental tumors compared to monolayer cultures. Drug sensitivity assays highlighted the model's translational relevance, as *BRAF*^{V600E} spheroids responded to combined dabrafenib and trametinib treatment in line with clinical outcomes (15). One of the challenges highlighted in this study was the difficulty in generating 3D structures from cells derived from highly aggressive tumor samples, such as ATC, which could be

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primarily explained by the elevated expression of epithelial-to-mesenchymal transition (EMT) markers. The downregulation of E-CADHERIN expression compromises cell–cell adhesion, thereby hindering the formation of spheroids. This lack of adhesion poses a significant obstacle in developing robust 3D models from such aggressive tumor samples. While the system demonstrated robust tumor mimicry and therapeutic insight, challenges such as long-term stability and scalability remain for future refinement.

An innovative and accessible fine-needle aspiration (FNA)-based technique for developing human PDTOs from a variety of tumor tissues, including TC, was also developed (49). FNA samples could be directly cultured without additional processing, enabling improved preservation of the tumor's original features, such as the tumor microenvironment and immune cell capture. Although the primary goal of the study was to describe a technique rather than focus on TC organoids, it provides PTC and ATC histopathological phenotypes. However, no differentiation status or long-term maintenance of thyroid organoid characteristics is provided (49). Recently, the same group, using the FNA method, described the generation of two PDTO models of ATC, one $BRAF^{WT}$ and another $BRAF^{V600E}$ mutant (50). These models were used to evaluate the therapeutic potential of pyrvinium pamoate, a potent Wnt/β-catenin signaling inhibitor. The authors described that treatment with the inhibitor significantly reduced tumoroid growth, in combination with standard TC treatment and alone. Notably, this method to derive TC organoids holds potential for patient-specific HTS, while carrying the advantage that FNA is a minimally invasive technique widely used as a first-line procedure for sampling and diagnosing TCs. However, taking into consideration the expected tumor cell heterogeneity, FNA-derived organoids from distinct areas of the tumor should be generated to explore therapeutic strategies.

In another demonstration of the clinical potential of PDTOs, Guo *et al.* (2025) conducted a prospective phase 2 study to guide personalized treatment in patients with locally advanced TC (44). Biopsies from primary tumors or metastatic sites from 75 patients with various TC histotypes were used to establish PDTOs and perform *in vitro* drug sensitivity testing. Based on these results, 55 patients received targeted therapies, achieving an overall objective response rate of 37.2%. In addition, 24 patients underwent surgical resection, with 19 achieving complete resection, suggesting that PDTO-based treatment choice may enhance operability and outcomes in advanced TC. This study is the first to use TC PDTOs to validate precision therapy improving patient prognosis.

In conclusion, PDTOs are useful models for studying TC *in vitro*, offering the opportunity to capture the complexity of each patient's tumor and helping to understand cancer behavior, treatment resistance, and responses to targeted therapies. PDTOs have been used to model both well-differentiated and aggressive types of TC, such as PDTC and ATC. While there are still some challenges – like inconsistent growth, short-term stability, and difficulty in modeling very aggressive tumors – combining PDTOs with genetic and drug testing tools makes them a promising approach for developing more personalized and effective treatments.

PSC-derived TC organoids

Contrary to PDTOs, PSC-derived organoids allow the study of the driver mutations effect and their mechanisms, early events associated with carcinogenesis, assessment of tumor behaviors, and the discovery of early markers for aggressiveness. Recently, by combining advances in genome editing and organoid technology, Venegas *et al.* (2025) developed a model to functionally study patient-specific *PTEN* variants associated with TC. Using CRISPR, the authors generated human iPSCs to analyze either the cancer-associated $PTEN^{M134R}$ mutation or the autism-associated $PTEN^{G132D}$ variant (36). The iPSC lines were differentiated into thyroid organoids using a modified version of Kurmann's protocol originally established for mESCs (51). Following gamma irradiation, both bulk and scRNAseq revealed that those organoids carrying the $PTEN^{M134R}$ variant exhibited strong activation of the p53 signaling pathway, resulting in an enhanced DNA damage response and increased apoptosis. In contrast, organoids with the $PTEN^{G132D}$ variant displayed a blunted p53 response, despite expressing comparable levels of PTEN protein. These findings suggest that the $PTEN^{M134R}$ variant may confer a gain-of-function effect on the p53-mediated stress response, independent of the canonical PI3K-AKT signaling. This model enables functional analysis of patient-specific *PTEN* variants in TC by using isogenic human iPSC lines for comparisons between variants and combining genome editing techniques with scRNAseq for high-resolutions mechanistic insights. Despite the relevance of the study, the results need to be taken within a context, since the protocol for thyroid organoid differentiation is adapted from a mouse system and may not fully mimic human thyroid maturation.

Aiming to study the early stages of TC development, a TC organoid model derived from hESCs was presented by Veschi *et al.* (2023) (19, 52). Using hESCs, they reproduced the protocol developed by Longmire *et al.* (2012), which achieved the development of thyroid progenitors after 25 days using mESCs in culture, using serum-free medium, and consecutive steps of induction using different growth factors (53). Briefly, in Veschi's study, they induced definitive endoderm in hESCs, generated thyroid progenitor cells, and promoted differentiation/maturation using a series of growth factors-supplemented media. Using the CRISPR/Cas9 system,

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specific combinations of oncogenic mutations (*BRAF*^{V600E}, *NRAS*^{Q61R}, *TP53*^{R248Q}) were introduced into thyroid progenitor cells. The authors observed that specific mutations resulted in distinct TC histotypes after cells were xenografted into the mouse avatar model. As expected, *BRAF*^{V600E} led to PTC features, *NRAS*^{Q61R} induced FTC, and the ATC phenotype was recapitulated when either *BRAF*^{V600E} or *NRAS*^{Q61R} was combined with *TP53*^{R248Q}. Using next-generation sequencing, the authors characterized the molecular mechanisms by which different TCs acquire aggressive traits over time, performing both genomic and transcriptomic analyses to examine gene expression and genetic alterations. They identified an EMT signature driven by the ternary complex TIMP1/MMP9/CD44 and KISS1R, which promoted tumor cell proliferation through the PI3K/AKT pathway. In addition, they observed that MMP9 activation coordinated with ERK activation to induce EMT via TWIST and SNAIL. This protocol enables the precise introduction of oncogenic mutations using CRISPR/Cas9 (52). The authors argue that their model demonstrates tumorigenesis originating from stem cells, proposing a developmental basis for cancer rooted in stem cells' self-renewal capacity and longevity, which theoretically allows for the accumulation of genetic mutations. However, this approach assumes stem cells as the primary origin of TC – a hypothesis that remains controversial. In support of the view that TC may not originate from a discrete stem cell population, it is informative to consider other cancers arising from tissues that, such as the thyroid, have a low cellular turnover and where regeneration is not driven by a classical, quiescent stem cell population. In organs such as the pancreas and prostate, cancer initiation has been linked to plasticity or metaplastic transitions from differentiated epithelial cells (54, 55). Similarly, in the prostate, luminal progenitor cells have been implicated in tumor initiation, particularly in castration-resistant disease (56). Thyroid regeneration is poorly understood and appears to occur without a dedicated stem cell population. Recent scRNAseq studies have failed to identify resident thyroid stem cells, suggesting that regeneration may occur through mechanisms independent of dedicated stem cells (28, 29). These observations raise the possibility that TC arises not from tissue-resident stem cells, as Veschi *et al.* hypothesize, but from mature follicular cells acquiring plasticity through oncogenic reprogramming, rather than from a stem-like population of cells. However, this remains speculative, as the earliest cellular and molecular events leading to thyroid carcinogenesis are not yet fully understood.

Similarly, our group used the mESC-derived thyroid organoids (57) to establish a TC organoid model using an inducible system for *Braf*^{V637E} mutation (the murine equivalent of human *BRAF*^{V600E}). This protocol utilizes a genetically engineered mESC line to time-specifically induce thyroid differentiation and *Braf*^{V637E} expression under tamoxifen stimulation (17). After thyroid differentiation, thyroid follicles full maturation and function, the *Braf*^{V637E} expression was induced by the addition of 4OHT, leading to activation of the MAPK pathway. The consequences of *Braf*^{V637E} expression were observed as early as 48 h post 4OHT addition, with downregulation of thyroid genes and disruption of follicular organization, indicating the initiation of a process of thyroid dedifferentiation. One of the major consequences of the *BRAF* mutation and MAPK pathway activation in TC is RAI refractoriness due to *sodium/iodide symporter* (*Nis*) downregulation. Several compounds previously described for re-establishing *Nis* expression were tested on the *Braf*^{V637E} organoid model. Remarkably, the combination of MEK inhibitor, PI3K inhibitor, and HDAC inhibitor (VPA) effectively restored *Nis* expression and its proper localization at the basolateral membrane of the follicle. In addition, treatment with drugs commonly used for advanced TC in patients, dabrafenib and trametinib, resulted in complete restoration of *Nis* levels. Remarkably, the combination of trametinib with the PI3K inhibitor led to a significant increase in key thyroid markers while restoring follicular morphology. Transcriptomic analysis confirmed the gene expression similarity of the PTC-derived organoids to expected findings in humans and the redifferentiation transcriptomic status promoted by the inhibitor treatments. Despite the promising PTC phenotype, this model is not suitable for long-term studies, since *Braf*^{V637E} expression is controlled by the Tg promoter, which is downregulated under dedifferentiation, causing instability of *Braf* oncogene expression.

Together, these studies reflect a significant advancement in the use of PDTO models and PSC-derived organoids and highlight the applications of these systems to study thyroid carcinogenesis (Supplementary Table 1 (see section on Supplementary materials given at the end of the article)). In addition, by enhancing the precision of preclinical models, these developments hold great potential for improving the prediction of therapeutic responses and accelerating the progression of personalized treatment strategies for TC patients. They also provide an important platform for the development of new therapies with a potential impact on patients' prognosis, specifically for the more aggressive TC subtypes (Fig. 1).

Challenges in developing more complex and physiological thyroid cancer organoid models

The various organoid models and aspects discussed here, such as early dedifferentiation and redifferentiation, techniques to develop avatars for personalized medicine, drug screening, and transcriptomic profiling,

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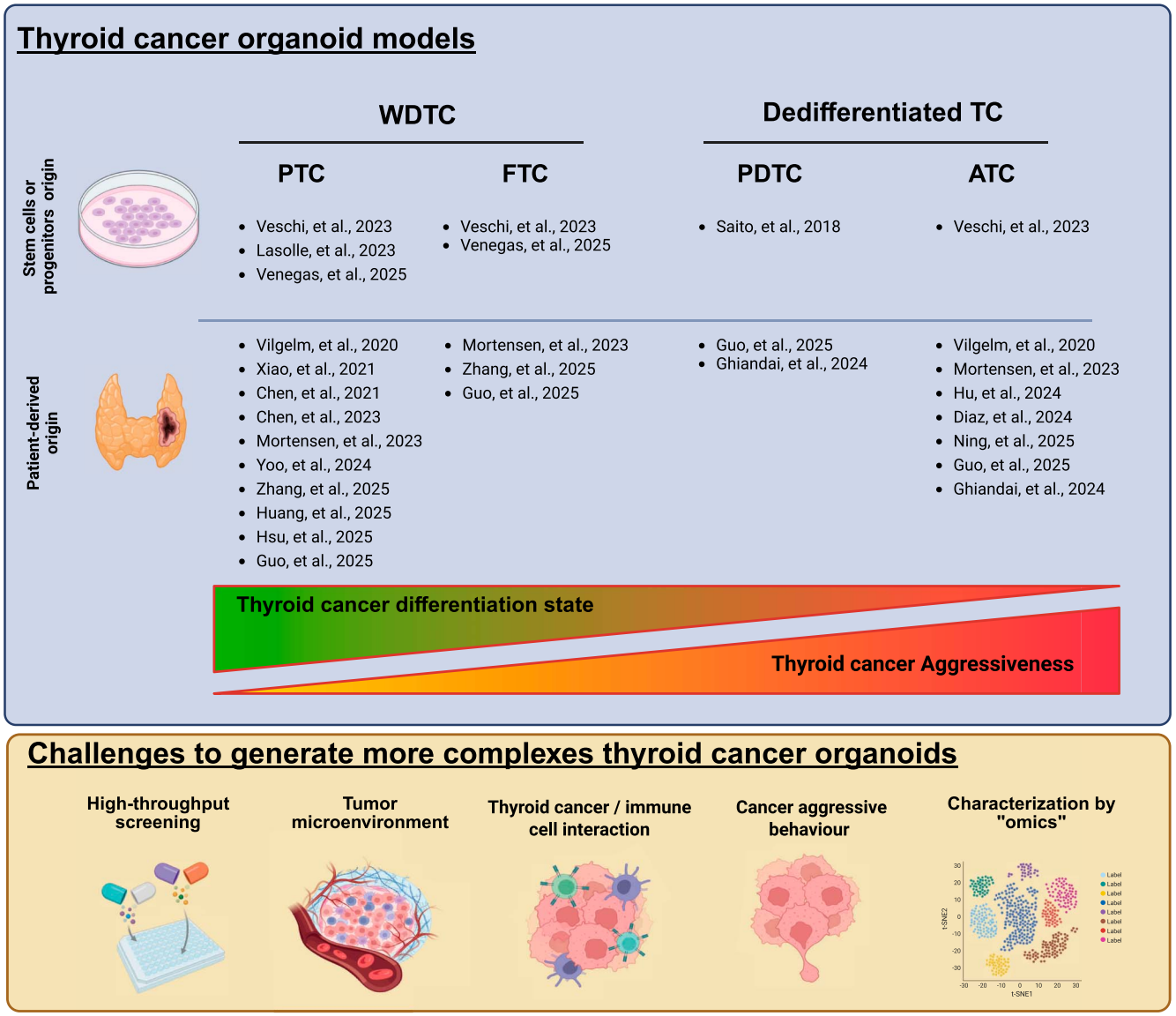

Figure 1

Overview of key TC organoid models and future challenges. This figure highlights significant TC organoid models, including those derived from human and mouse pluripotent stem cells, tissue progenitors, and patient-derived tumor samples. These models enable the study of various TC types, offering valuable insights into cancer mechanisms and supporting the development of enhanced prognostic strategies and therapies. Progress in TC research will rely on leveraging innovative methodologies to address current limitations and achieve transformative advancements while generating more complex models.

hold significant relevance for the study of TC. Both PSC-derived organoids and PDTOs serve as valuable tools to investigate these processes, with some specificities and strengths depending on the question to be addressed. These models have laid a strong foundation in the understanding of TC biology and for future research developments. However, insights from organoids derived from other tissue types may offer valuable lessons and new perspectives. For instance, studies have addressed critical aspects not yet extensively explored in current TC organoid models, such as drug resistance molecular mechanisms, tumor invasion, metastasis, and the TME (Fig. 1).

Tumor microenvironment

As exemplified above, TC organoids hold significant promise and offer a novel approach to advancing biological and translational clinical research. However, some limitations must be addressed to closely mimic physiological conditions. Currently, most TC organoid models lack TME components, which play critical roles

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in cancer progression and prognosis *in vivo* (58). Cancer is not merely a mass of proliferating cells; it also incorporates a range of non-cancerous cells that contribute to its survival and progression (59). Indeed, there is a bidirectional communication between cancer cells and cellular components of their surrounding microenvironment. The microenvironment of TC is composed of immune cells, such as myeloid cells, natural killer cells (NK), T and B cells, as well as stromal cells such as endothelial cells and cancer-associated fibroblasts (CAFs) (58). In patients with TC, the immune cells have been shown to be associated with advanced forms of cancer, promoting disease severity, undifferentiated states, cell survival, therapeutic resistance to targeted drugs, and metastasis (58). Similarly, *in vivo* studies demonstrated the recruitment of CAFs into the microenvironment of *Braf*^{V600E}*/Pten*$^{-/-}$*/TPO-Cre* TC in mice. CAFs accumulation led to increased deposition of collagen I in the ECM of the cancer. *In vitro*, the *Braf*^{V600E}*/Pten*$^{-/-}$*/TPO-Cre* TC cells cultured in collagen I-coated plates have shown an increase in motility compared to the condition without collagen I precoating (60). Given the importance of TME in TC, new methods to establish TC organoids should focus on preserving or reconstituting this TME to produce a model as close as possible to the *in vivo* context.

Several approaches nowadays can be used to reconstruct the TME of cancer organoids, such as the co-culture of multiple cell types in the same microenvironment (61). To date, no study in the literature has applied this approach to preserve the cellular microenvironment of TC organoids. Nevertheless, the method has been developed and successfully applied in other cancer types, demonstrating considerable potential. For instance, PDTOs from resected colorectal cancer typically lack CAFs, which constitute a significant portion of the TME *in vivo* and play a vital role in cancer progression and drug resistance. To mimic this aspect, Luo *et al.* (2020) developed a co-culture model integrating PDTOs with patient-derived CAFs. In this system, CAFs helped to sustain the proliferation of PDTOs in growth factor-free media and restored pathways that are active in patient tissues but absent in PDTOs cultured alone. When treated with standard-of-care drugs, PDTOs co-cultured with CAFs exhibited greater drug resistance compared to PDTOs cultured alone (62). Along the same lines, efforts to incorporate immune components into organoid cultures have also proven effective in recapitulating aspects of the TME. Dijkstra *et al.* (2018) developed a co-culture system designed to expand and isolate tumor-reactive T cell populations. Their approach involved co-culturing peripheral blood mononuclear cells (PBMCs) with autologous PDTOs derived from mismatch repair-deficient colorectal and non-small cell lung cancers. To enhance tumor antigen presentation, PDTOs were pre-treated with interferon-gamma (IFNγ), and only MHC class I-proficient cancer organoids were selected to ensure efficient T cell engagement. After 2 weeks of co-culture, the PBMCs demonstrated robust expansion of tumor-reactive CD8$^+$ T cells, as evidenced by IFNγ secretion and CD107a upregulation. When re-exposed to PDTOs, these tumor-reactive CD8$^+$ T cells induced a substantial reduction in organoid size and widespread apoptosis, demonstrating their ability to suppress carcinogenesis (63). Immunotherapy in the context of TC organoids opens opportunities for exploring the interplay between TC cells and the immune system. For instance, organoid-based HTS could facilitate the discovery of immunotherapy agents that target immune escape mechanisms, such as defective antigen presentation or resistance to immune checkpoint blockade. It could also allow the evaluation of novel immunotherapeutic combinations, such as immune checkpoint inhibitors, in patient-specific contexts. This synergy between HTS and immunotherapy, using TC organoids, holds the potential to accelerate the development of more effective, personalized therapies.

Tumor vascularization

The lack of vascularization in organoids remains a major challenge, limiting their long-term culture, growth, and maturation (64). When organoids are cultured for extended periods or reach a certain size, the cells at their core often undergo apoptosis or necrosis due to inadequate oxygen and nutrient supply, as well as difficulty in removing metabolic waste (64). To address this issue, various strategies for vascularizing organoids have been developed, including co-culturing organoids with endothelial cells (65), transplanting organoids into animal models (66), or employing bioengineering techniques such as microfluidic organ-on-chip (OoC) platforms (67). To vascularize iPSC-derived whole-brain organoids, researchers employed a co-culture with iPSC-derived endothelial cells from the same patient. After 20 days of co-culture, the brain organoids exhibited a robust vascular network, primarily in the outer layers, with some blood vessels infiltrating the core of the organoid (65). In another approach, a platform called 'Organ-On-VascularNet' has been developed. The key component of this system involves mature endothelial cells that transiently express the ETS variant transcription factor 2 (ETV2), a protein normally found in embryonic endothelial cells but absent in adults. The transient expression of ETV2 reprograms mature ECs into more flexible, vasculogenic cells (R-VECs). When co-cultured with patient-derived colorectal cancer organoids and 'healthy' colorectal organoids (COs), R-VECs supported vascular network formation throughout the matrix, with a larger vascular area than when patient-derived colorectal cancer organoids and COs were co-cultured with adult ECs. scRNAseq of R-VECs co-cultured with colorectal cancer organoids, COs, or cultured alone revealed distinct gene expression patterns specific to each culture condition,

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suggesting that R-VECs adapt to their microenvironment and external stimuli (68).

Tissue dynamics

While the organoid culture systems mentioned above have addressed key limitations in cancer organoid development, such as the absence of a TME and vascularization, these *in vitro* models remain static and do not facilitate the study of several phenomena, such as the recruitment of stromal and immune cells, invasion, and metastasis. The static nature means they lack essential mechanical forces such as fluid shear stress, hydrostatic pressure, and tissue deformation, all of which significantly influence cancer cell behavior *in vivo* (69). The advent of microfluidic OoC technology has helped overcome these challenges by introducing dynamic flow and enabling the incorporation of shear forces and mechanical strain (69, 70). Microfluidic platforms operate by manipulating small volumes of fluids within networks of microscale channels containing living cells to model the physiological functions of tissues and organs (70, 71). The goal of these devices is not to create a fully functioning organ but to build a reductionist model that recapitulates organ function and structure as closely as possible to the *in vivo* situation (70). If applied to TC research, this technology can help to provide interesting insights into TC pathogenesis and progression in a more physiologic environment. Riley *et al.* (2019) introduced a novel microfluidic device capable of preserving the viability and functional integrity of malignant thyroid tissue slices for at least 4 days. This platform enabled real-time assessment of tissue responses to therapeutic agents, such as etoposide (topoisomerase II inhibitor) and SP600125 (JNK inhibitor), demonstrating its potential for drug screening and personalized medicine applications (72). Despite these developments, there are currently no reports on the application of OoC technology in TC organoid research. Nevertheless, several studies have employed such platforms for non-malignant thyroid research. Notably, Carvalho *et al.* (2023) developed a thyroid organoid-on-a-chip system to investigate the effects of endocrine disruptors on TH biosynthesis. In this study, mESC-derived thyroid organoids were cultured in a microfluidic device for 7 days, and more than 50% of the follicles produced thyroxine (T4), in contrast to only 1% under static conditions. Furthermore, exposure of OoC thyroid organoids to the endocrine disruptor benzo[k] fluoranthene (BKF) induced significant alterations in thyroid gene expression profiles, leading to the downregulation of key genes involved in TH synthesis (73). These findings highlight the improved physiological relevance of microfluidic systems. In a complementary effort, Kühnlenz *et al.* (2023) developed a co-culture OoC model integrating functional human thyroid organoids and liver spheroids. The patient-derived thyroid organoids were able to synthesize T3, while the hepatic spheroids contained the full enzymatic and transporter machinery required for TH metabolism. The two organ models were maintained in distinct but fluidically connected compartments of the microfluidic device (74). This integrated thyroid–liver (OoC) system offers a physiologically relevant platform to study inter-organ interactions and illustrates the feasibility and relevance of thyroid-on-chip technologies for addressing key questions in thyroid carcinogenesis, including cell migration and metastatic potential.

In the realm of other types of cancer, the microfluidic system has been successfully applied for various types of cancers and has shown promise (69). Hassell *et al.* (2017) engineered a microfluidic OoC platform to simulate the physiological lung microenvironment for non-small-cell lung cancer (NSCLC), facilitating the investigation of microenvironment-dependent tumor proliferation, induction of dormancy, and differential responses to tyrosine kinase inhibitor therapies, thereby closely recapitulating *in vivo* patient-specific disease dynamics. They found that simulated breathing motions significantly reduced tumor cell proliferation, invasion, and sensitivity to the EGFR inhibitor rociletinib compared to static conditions. These observations suggest that the mechanical forces associated with respiration play a critical role in modulating tumor progression and drug responsiveness, and that the loss of lung elasticity, potentially due to tumor burden and alveolar occlusion, may contribute to disease advancement in NSCLC (75). This study underscores the utility of OoC technologies as physiologically relevant *in vitro* platforms for elucidating complex tumor–microenvironment interactions and for advancing preclinical assessment of anticancer therapeutics.

Conclusion

Organoid technology has emerged as a transformative tool in TC research, offering new insights into aggressive subtypes such as PDTC and ATC that have traditionally been challenging to study and treat. The integration of organoid models with HTS platforms and ‘omics’ technologies represents a particularly promising avenue for advancement. As demonstrated in other cancer types, these combined approaches can elucidate the complex molecular mechanisms underlying aggressive TCs, potentially uncovering novel therapeutic targets. Furthermore, by correlating genomic profiles with drug sensitivity data, these methods facilitate the development of precision medicine strategies. The application of these methodologies to patient-derived organoid models is proving instrumental in drug discovery and personalized treatment approaches, positioning organoid technology at the forefront of TC research and clinical innovation.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/ETJ-24-0392.

Declaration of interest

SC and MR have filed an international patent regarding the hESC-derived thyroid organoids.

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